IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicants:	Gregory J. LaRosa and Walter Newman		
Application No.:	09/898,513	Group:	1648
Filed:	July 3, 2001	Examiner:	A. Salimi
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For: ANTI-CCR2 ANTIBODIES AND METHODS OF USE THEREFOR

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on 5/8/03 (Date) Paula DePeltteau (Signature)

PAULA DEPELTEAU
Typed or printed name of person signing certificate

DECLARATION UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Gregory J. LaRosa, of 325 Woodward Street, Newton, MA 02468, and Walter Newman, of 3 Durham Street #3, Boston MA 02115, declare and state that:

1. We are the inventors of the above-identified patent application. This application is a continuation application of U.S. Serial No. 09/121,781, filed July 23, 1998 (now U.S. Patent No. 6,312,689) and is assigned of record to Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02141.

2. An antibody which binds to a mammalian CC-chemokine receptor 2 and inhibits binding of a ligand to the receptor was conceived by us prior to the effective date of the following references and diligently pursued from prior to said effective date to a subsequent reduction to practice of the invention in the United States. The references are:

a) U.S. Patent No. 6,084,075 to Lind *et al.* (effective date of February 28, 1997);

b) Frade *et al.* (*J. Clin. Invest. 100*(3):497-502 (1997) (effective date of August 1, 1997);

c) Frade *et al.* (*J. Immunol. 159*(11):5576-5584 (1997) (effective date of December 1, 1997); and

d) WO 97/31949 to Lind *et al.* (effective date of September 4, 1997).

3. The statements made in paragraph (2) are shown by the attached Exhibits A-L. In accordance with accepted U.S. Patent Office practice, the dates on Exhibits A and B, submitted to evidence conception of the claimed invention, have been redacted.

Exhibit A is copies of selected pages from a Research, Development and Marketing Agreement (Agreement) between LeukoSite, Inc. and Warner-Lambert Company. In December of 1999, LeukoSite, Inc. was acquired by Millennium Pharmaceuticals, Inc. as a wholly owned subsidiary. On March 15, 2000, a Certificate of Ownership and Merger merging LeukoSite, Inc. into Millennium Pharmaceuticals, Inc. was executed, and the merger became effective on March 16, 2000. Exhibit A is dated prior to February 28, 1997.

Pages A-1 and A-2 and A-3 are the first page and last (signature) pages, respectively, of the Agreement. Pages A-4 through A-10 are Exhibit I of the Agreement and provide an overview of the collaborative research plan. The highlighted portion of page A-6 evidences our intent to develop a blocking antibody to MCP-1 receptors A and B (known as CCR2A and CCR2B). The highlighted portion of page A-8 evidences the protocol by which we reasonably expected to produce blocking antibodies, i.e., raising antibodies against N-

terminal peptide fragments and subsequently against murine cells expressing high levels of receptors. Page A-9 evidences our plan to utilize an MCP-1 ligand binding assay to identify antibodies with receptor antagonist properties (i.e., antibodies which inhibit binding of ligand to the CCR2 receptor).

Exhibit B is a copy of Greg LaRosa's yearly Goals and Objectives and Performance Planning dated prior to February 28, 1997. This document describes Dr. LaRosa's goals and objectives for the year and the status of his work in those areas. The highlighted portions of pages B-1 and B-2 evidence Dr. LaRosa's coordination of the work on the generation of cell lines stably transfected with CCR2 for use as immunogen to generate monoclonal antibodies which bind CCR2, assessment of the anti-CCR2 antibodies already generated at that time with regard to affect on ligand binding to receptor, and generation of additional anti-CCR2 antibodies using murine cell lines stably transfected with CCR2. The highlighted portion of page B-3 evidences the status of the work, particularly with regard to the generation of CCR2 constructs for use in generating stable receptor transfectants and testing of anti-CCR2 antibodies for binding to transfectants. Specifically, page B-3 evidences that several constructs had been generated which express native CCR2, and two antibodies have been tested for and demonstrated binding to cells transiently transfected to express CCR2. In addition, anti-CCR2 antibody 5A11 had been purified. On page B-4, in addition to the date, Dr. LaRosa's Social Security Number has also been redacted.

Exhibit C is a copy of a page from Nasim Kassam's notebook dated February 18, 1997 and evidencing a fusion of spleen cells from a mouse immunized as shown with SP2/0 cells to produce hybridomas. The mouse was immunized on November 18, 1996, November 26, 1996, December 11, 1996, December 31, 1996, January 10, 1997, January 24, 1997 and February 13, 1997 with the immunogen as shown. This periodic boosting of the mouse with immunogen was performed to generate a vigorous immune response in the mouse. On February

16, 1997, spleen cells from this mouse were fused with the immortal cell line SP2/0 to produce hybridomas as a part of the continued work to generate antibodies which bind CCR2.

Exhibit D is a copy of a page from Nasim Kassam's notebook dated May 1, 1997 and evidencing a fusion of spleen cells from a mouse immunized as shown with SP2/0 cells to produce hybridomas. The mouse was immunized on November 8, 1996, November 15, 1996, November 26, 1996, December 12, 1996, December 27, 1996, January 10, 1997, January 23, 1997, February 13, 1997, February 20, 1997, March 6, 1997, April 10, 1997 and April 28, 1997 with either CCR2 peptide (PPD #2) or B-10 cells transfected with CCR2 as immunogen as shown. This periodic boosting of the mouse with immunogen was performed to generate a vigorous immune response in the mouse. On May 1, 1997, spleen cells from this mouse were fused with the immortal cell line SP2/0 to produce hybridomas as a part of the continued work to generate antibodies which bind CCR2.

Exhibit E is a copy of a page from Nasim Kassam's notebook dated August 18, 1997 and evidencing a fusion of spleen cells from a mouse immunized as shown with SP2/0 cells to produce hybridomas. The mouse was immunized on November 8, 1996, November 15, 1996, November 26, 1996, December 12, 1996, December 27, 1996, January 10, 1997, January 23, 1997, February 13, 1997, February 20, 1997, March 6, 1997, April 10, 1997 and August 12, 1997 with either CCR2 peptide (PPD #2) or B-10 cells transfected with CCR2 as immunogen as shown. This periodic boosting of the mouse with immunogen was performed to generate a vigorous immune response in the mouse. On August 15, 1997, spleen cells from this mouse were fused with the immortal cell line SP2/0 to produce hybridomas as a part of the continued work to generate antibodies which bind CCR2.

Exhibit F is copies of pages from Amy Reinhart's laboratory notebook dated September 5, 1997 which evidence the transfection of murine L1/2 cells with the DEF3 plasmid (CCR2b.DEF3) and the IRES plasmid, each containing the nucleotide sequence of CCR2, to produce cell lines stably transfected with CCR2. The transfection protocol was performed on September 5, 1997 and September 8, 1997. These pages demonstrate continued work to generate cell lines stably transfected with CCR2 for use, *inter alia*, as an immunogen for antibody generation.

Exhibit G is copies of pages from Amy Reinhart's laboratory notebook which show the selection on September 24, 1997 of cells transfected with CCR2b on the basis of their ability to chemotax in response to MCP-1. Cells able to chemotax in response to MCP-1 as compared with an untransfected control were selected as stably transfected with CCR2. In addition, on October 9, 1997 and October 24, 1997, cells transfected with CCR2b were stained with anti-CCR2 monoclonal antibody 5A11 to identify cells that expressed CCR2b. Page G-2 shows the dot plots and histogram plots generated as described in the protocols performed on October 9 and 24, 1997. Pages G-3 and G-4 show the settings of the FACS machine used in the work shown. Exhibit G demonstrates the continued work to generate cell lines stably transfected with CCR2 for use, *inter alia*, as an immunogen for antibody generation.

Exhibit H is a copy of a page from Amy Reinhart's laboratory notebook dated, *inter alia*, November 19, 1997, December 4, 1997, December 10, 1997 and December 19, 1997 showing the preparation of cells transfected with a CCR2b.DEF3 vector to produce cells expressing CCR2b for immunization and showing the status of the immunized mice.

Exhibit I is a copy of a page from Nasim Kassam's laboratory notebook dated December 22, 1997 and evidencing a fusion of spleen cells from a mouse

immunized as shown with SP2/0 cells to produce hybridomas. The mouse was immunized on November 8, 1996, November 15, 1996, November 26, 1996, December 12, 1996, December 27, 1996, January 10, 1997, January 23, 1997, February 13, 1997, February 20, 1997, March 6, 1997, April 10, 1997, November 6, 1997, November 19, 1997, Decmeber 4, 1997 and December 19, 1997 with either B-10 cells transfected with CCR2, CCR2 peptide (PPD #2) or cells transfected with the CCR2b.DEF3 vector as immunogen as shown. On December 22, 1997, spleen cells from this mouse were fused with the immortal cell line SP2/0 to produce hybridomas as a part of the continued work to generate antibodies which bind CCR2. This fusion (LS-132) gave rise to the hybridomas which produced the 1D9 and 8G2 antibodies described in the subject application.

Exhibit J is a copy of a page from Amy Reinhart's laboratory notebook dated December 30 and 31, 1997 which shows an assessment of the staining of cells transfected with CCR2 with the supernatants from the LS-132 fusion prepared as shown in Exhibit I. The wells for 1D9 and 8G2 were positive, indicated by the fact that they stained CCR2-transfected cells.

Exhibit K is copies of pages from Amy Reinhart's laboratory notebook dated January 2, 1998 showing further analysis of supernatants from the LS-132 fusion and confirming wells 1D9 and 8G2 as positive. Specifically, these results confirmed that antibodies produced by the 1D9 and 8G2 hybridomas bind cells transfected with CCR2 but did not bind wild type (untransfected) cells.

Exhibit L is copies of pages from Amy Reinhart's laboratory notebook dated January 9, 1998 showing that monoclonal antibodies produced by, *inter alia*, hybridoma LS-132-1D9 were able to inhibit binding of MCP-1 to murine L1/2 cells transfected with CCR2. These results confirmed that the monoclonal antibodies produced by this hybridoma bind to CCR2 and inhibit binding of a ligand to CCR2.

4. Amy Reinhart and Nasim Kassam performed the work described in the notebook records described herein under our direction and supervision.

5. These facts show that an antibody which binds to a mammalian CC-chemokine receptor 2 and inhibits binding of a ligand to the receptor was conceived by us prior to February 28, 1997 as evidenced by Exhibits A and B submitted herewith, and diligently pursued from prior to February 28, 1997 to a subsequent reduction to practice of the invention by us in the United States as evidenced by Exhibits C-L submitted herewith.

We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that willful false statements may jeopardize the validity of any patent issuing on the subject application.

Gregory J. LaRosa

5/7/03

Date

Walter Newman

5/7/03

Date